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Prospects and challenges in soya component allergy study: a systematic literature overview

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Abstract

The study aims to conduct a comparative analysis of the data available in the current literature on soybean allergens and evaluation of interventions aimed at attenuating their immunogenic potential. The research relevance is determined by the escalation of the frequency of food hypersensitivity against the background of a global trend towards increased consumption of soy derivatives. The research methodology was based on a comprehensive analysis of current scientific data on the immunogenic properties of key soybean allergens, in particular β-conglycinin and glycinin, and their processing methods, and includes an analysis of 53 papers by authors from around the world. A comparative evaluation of the effectiveness of various processing methods, including thermal modification, ultra-high-pressure application and enzymatic hydrolysis, aimed at reducing the antigenic characteristics of soy proteins was carried out. The study integrated data from clinical trials and experimental results reflecting the structural modifications of protein molecules after treatment. The analysis of the data shows that the allergenic properties of soybeans are mainly due to glycine, β-conglycinin and their derivatives. These proteins are sufficiently thermostable and resistant to enzymatic processing, which makes their use in isolation ineffective. The combined use of enzymatic treatment and ultra-high pressure is most effective in reducing the allergenic potential of soy proteins, but the most promising is the use of specific breeding methods. Moreover, the study confirmed the significant potential of genetic engineering methods aimed at reducing the expression of allergenic proteins in soybean crops, which opens new prospects for the prevention of allergic reactions.

Keywords: Hypersensitivity. Atopy. Food intolerance. Sensitisation. Biomodification.

Introduction

Allergic reaction to soya products is an increasingly important problem in the field of food allergology, especially in light of the rapidly increasing consumption of soya in the food industry and in people's daily diets. The importance of the study of this disease stems from the fact that soya allergy, in all its clinical polymorphism, can manifest in a wide range of nonspecific manifestations, from mild skin rashes to severe systemic disorders such as anaphylactic shock. These aspects emphasise the critical importance of an in-depth study of the immunological features of soybean allergy. With the global trend towards plant-based diets and increasing production of soya products, this problem is of particular relevance and requires the attention of both medical specialists and food technology researchers **[1]**.

Epidemiological data of recent years, presented in the works of Spolidoro et al. **[1,2]**, indicate that sensitisation to food allergens in Europe has reached 7.5-19%. Despite its growing prevalence, soy allergy often remains out of the focus of European experts, being in the shadow of the so-called "Big Eight" allergens that are more common in the region. This fact makes it difficult to assess the true scale of the problem and highlights the need for a more detailed study of pathogenetic mechanisms and the development of

effective methods to prevent contact with products of this group. The presented research papers are based on the study of the prevalence and manifestations of the problem while ignoring the causes and ways of preventing it.

Studies conducted by Kuźmiński et al. **[3]** addressed soy proteins as triggers of severe allergic reactions in children, including such conditions as allergic colitis and eosinophilic gastroenteritis. These diseases are characterised by a pronounced inflammatory response of the gastrointestinal mucosa, which may become a determinant that potentiates a cascade of delayed morphological and functional deviations in terms of somatic and psychophysiological ontogenesis. The obtained data emphasise the critical importance of timely identification of the problem, as well as the need to exclude soy products from the diet of patients with confirmed hypersensitivity. However, it should be noted that the complete elimination of such a broad category of products is not an acceptable solution in the medium to long term, and research should be focused on product modification rather than elimination.

One of the fundamental areas requiring in-depth scientific research is the influence of environmental factors on the formation of food allergies. The works of Sikorska-Szaflik and Sozańska **[4]**, and Kuźniar et al. **[5]** pay special attention to the importance of changes in the microbiological profile of the gastrointestinal tract initiated by various environmental influences, including increased presentation of certain allergens. As was also pointed out by Tuzimski and Petruczynik **[6]**, sensitisation to food allergens is possible through contact with cosmetics. These factors have a multifaceted impact on the mechanisms of immune reactions, which leads to the formation of threatening trends in the expansion of food allergies, in particular, an allergic reaction to soy. Despite the comprehensive coverage of the problem, in the course of their work, the above-mentioned researchers faced the problem of the imperfection of existing knowledge regarding the trigger of an inadequate immune response and ways to modify it.

Despite significant advances in the understanding of general immunological reactions and improved methods of diagnosing soya product allergy, there are still numerous unresolved issues related to the aetiology, prevention and effective correction of this pathological condition. Modern scientific research is focused on symptom control, the use of bioactive compounds and the elimination of allergens from the diet. However, the aspects of preventing the development of allergic reactions remain incompletely addressed. Additional basic and clinical research is needed to develop more effective preventive measures that can reduce the incidence of soybean antigen exposure in sensitised individuals. Traditional measures seek to prevent food allergies by avoiding specific foods, which can lead to an unbalanced diet and is not preferable, which is a prerequisite for the development of new methods of processing the Big Eight products **[1,2]**.

The main objective of this review was to systematise and critically analyse the current information on the precursors of allergy to soy products. Particular emphasis is placed on the study of the mechanisms of disease pathogenesis, including a detailed research of allergenic soy proteins, such as glycine and β-conglycinin, and their interaction with immune system components. In addition, the review discusses modern methods of processing and modification of soy products aimed at reducing their allergenicity. This includes heat treatment, fermentation, protein hydrolysis, and the use of genetic engineering technologies to develop hypoallergenic soy varieties.

Methods

The present study is based on the results of an extensive bibliographic analysis of scientific literature aimed at forming a holistic view of modern concepts in the field of soy protein allergenicity and methods of their structural modification to reduce the antigenic potential. The central aspects of the bibliographic search were a detailed study of the structural and functional characteristics of key soy allergens, in particular, glycine and β-glycine; comparative analysis of the effectiveness of various methods of soy protein processing, including thermal modification, ultra-high-pressure application and enzymatic hydrolysis. To ensure maximum coverage of scientific publications, a search strategy was developed using several keywords: "soy allergens", "β-conglycinin", "glycin", "soy protein structure", "allergenic proteins", "immune response to soy", "IgE binding" – for publications on the immunogenicity of soy components and "protein processing", "thermal modification", "ultrahigh pressure", "enzymatic hydrolysis", "allergenic protein reduction", "protein modification techniques" – for publications on their modification.

To guarantee the relevance, validity and applied value of the selected scientific publications, the inclusion and exclusion criteria for sources were formulated and implemented within the framework of this study. The temporal range was narrowed to select publications written in the period from 2019 to 2024, which ensured focus on the most relevant and innovative data. The body of literature analysed included large-scale meta-

analyses and original empirical studies. Works published in leading peer-reviewed journals were selected to ensure a high level of scientific rigour and reliability of the data. The study integrated works conducted by research teams from different regions and states, which ensured the socio-economic diversity of research contexts and a wide range of geographical coverage. The implementation of these criteria made it possible to evaluate the effectiveness of soy protein processing and allergenic potential reduction methods in the context of different conditions characteristic of divergent food systems and production methodologies and to focus on the latest and most relevant scientific evidence based on rigorous methodological principles. Papers with insufficiently detailed descriptions of methodology that could compromise the reproducibility of the results were eliminated.

Papers with inadequate sample sizes that did not ensure the statistical significance of the results for extrapolation to wider populations were excluded. Publications that had not been peer-reviewed in recognised scientific journals, including preprints, were excluded. Studies focusing on aspects of soya proteins not directly related to their allergenic potential or methods of allergen reduction were excluded. Papers presenting secondary analyses of data already included in the study through primary sources were avoided to avoid artificial amplification of individual results. Particular attention was devoted to identifying and excluding papers with undeclared or significant conflicts of interest, as well as studies whose results were based on the use of outdated protein process methods.

This approach ensured the formation of a reliable and up-to-date knowledge base necessary for a comprehensive analysis of the problem of soya protein allergenicity and the development of innovative strategies to minimise it. The application of this methodology was used to accumulate and systematise a database of 53 publications on the allergenic properties of soy proteins and innovative methods of their modification to minimise the allergenic potential.

Results and Discussion

Main proteins-precursors of allergic reactions to soya products

Food hypersensitivity reactions are a special type of allergic response and can be classified into the following categories depending on their mechanism of development: immunoglobulin E (IgE)-mediated allergic reactions, reactions without IgE, and mixed forms involving both IgE and alternative immune pathways **[7,8]**. The most common food allergies are caused by IgE-mediated hypersensitivity reactions. The mechanism of sensitisation in such cases includes three key stages: the sensitisation phase, the activation phase and the effector response phase.

Upon initial contact with a food allergen, the immunocompetent system is activated, initiating the biosynthesis of highly specific IgE antibodies against a particular antigen **[9]**. These molecular mediators of allergy associate with the membrane receptors of labrocytes, which puts the body into a state of sensitisation. With repeated exposure to the same allergen, the activation stage occurs: IgE antibodies bind to the allergen, stimulating the release of various proinflammatory bioagents (including components of the kallikrein-kinin system and histamine) and the synthesis of new biologically active substances, including platelet-activating factor, prostaglandins and leukotrienes **[10]**.

The immunologically mediated excretion of biologically active substances initiates a cascade of reactions culminating in the effector phase of the allergic process **[11]**. This stage is characterised by the activation of local or systemic hypersensitivity reactions, which are realised through the action of mediators on a wide range of organs and tissues. The pathophysiological picture includes a triad of symptoms: tonic contraction of smooth muscle structures, hyperactivation of the secretory apparatus and increased vascular endothelial barrier capacity. These phenomena are a direct consequence of the liberation of vasoactive and proinflammatory agents during the immune response.

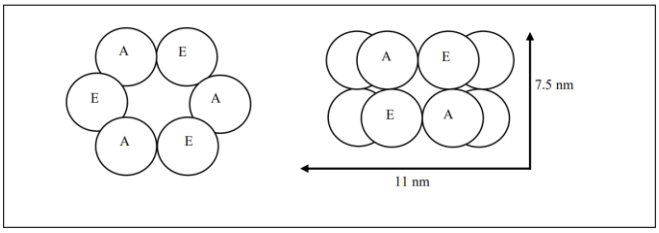
Hypersensitivity reactions not mediated by IgE are usually associated with the involvement of various cellular components and are most manifested in the digestive system. Their mechanisms and time course are not fully understood; such reactions are often misdiagnosed and referred to as delayed immune responses **[12,13]**. Food allergies can affect many organs and systems, posing a potential life-threatening threat and significantly impairing the quality of life of patients. Therefore, the issue of methods to reduce or eliminate the potential allergenicity of food proteins is attracting increasing attention.

Soybean allergens are high molecular weight proteins or glycoproteins found in soybeans and their products that can cause sensitisation reactions in humans, as well as in farm animals and birds. They are divided into four main types based on their sedimentation coefficients: 2S, 7S, 11S and 15S **[14]**. To date, 44 different types of soybean allergenic proteins are known. Among them, β-conglycinin (7S), glycinin (11S), Gly m Bd 28K and Gly m Bd 30K (also known as P34) are of particular importance, which are the main components of soy allergens that cause

hyperimmune reactions in the body. Glycine is a heterogeneous protein with a molecular weight in the range of 320 to 360 kDa, making up one-fifth to one-quarter of the structure of soybean proteins and about 40% of the total number of its globulin structures **[15]**.

Its molecular configuration is shown in Figure 1. Structurally, glycine is a hexamer consisting of two trimers. Each of these trimers is formed by different subunits connected by disulfide bonds between one acidic subunit (molecular weight 35-43 kDa, isoelectric point pI 4.8-5.5) and one alkaline subunit (18-20 kDa, pI 6.5-8.5). The total isoelectric value of glycine is approximately 6.4 **[16]**.

Figure 1. The molecular configuration of glycine.

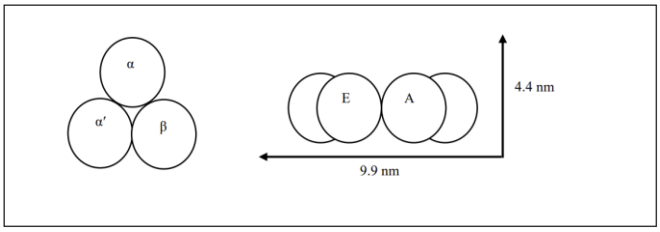


Source: compiled by the author.

The molecular weight of β-conglycinin varies from 150 to 200 kDa. This protein is a trimeric glycoprotein formed by hydrophobic interactions between different forms of three subunits: α′, α and β. The biophysical characterisation of the subunit composition revealed the following pattern of molecular weight distribution: the α′ component shows variability in the range of 57000-83000 Da, the α subunit ranges between 57000 and 76000 atomic mass units, while the β element is limited to the range of 42000-53000 Da. Their isoelectric values are 4.9, 5.18 and 5.66-6.00, respectively **[17]**.

The structure of β-conglycinin is illustrated in Figure 2. The allergenic properties of β-conglycinin are due to its subunits, especially α and α′. In particular, the α-subunit (also known as Gly m Bd 60K) has the highest allergenicity and is characterised by a low allergic reaction threshold; even minimal doses can provoke an allergic reaction **[18]**. All three subunits of β-conglycinin contain glycosyl groups, which gives them increased hydrophilicity and a more flexible spatial structure compared to 11S-globulin **[19]**.

Figure 2. Molecular configuration of β-conglycinin.



Source: compiled by the author.

After purification, it was found that all three subunits of β-conglycinin – α′, α and β – were able to bind to IgE in the blood of patients with soybean allergy, confirming their allergenicity **[20]**. The allergenic epitopes that bind to immunoglobulin G (IgG) were identified on the α-subunit **[19]**. Using immunoinformatic methods, the presence of 15 antigenic sites on the α-subunit that can bind to the above-mentioned class of immunoglobulins was predicted, of which 11 were confirmed as major allergenic epitopes closely associated with the secondary spatial organisation of peptide sequences **[21]**.

Recent studies have demonstrated that the peptide fragment ^{488}PHFNSKAIVVLV^{499} in the α′-subunit contains immunoreactive molecular regions characterised by both sequential and three-dimensional structural organisation. They can be destroyed by heat treatment **[22]**.

Similarly, using immunoinformatic methods, it was predicted that the β-subunit contains 10 linear epitopes that can bind to IgG in the serum of patients with soybean allergy, of which 5 epitopes can interact with IgE. Their amino acid sequences are as follows: ^{59}FNKRSPQLENLRDYR^{73},^{110}NDDRDSYNLHPGDAQRIPAG^{129}, ^{150}IPVNKPGRYDDFFLS^{164}, ^{197}FGEEEEQRQQEG^{208}, ^{225}AKSSSRKTISSEDEPFNLRSRNPIYS^{250} **[23]**.

The essential identity of amino acid sequences between the subunit triplets generates the possibility of cross-immunological phenomena, which necessitates the use of a differential approach in the autonomous analysis of individual components to exclude artefacts induced by this phenomenon **[24]**. Gly m Bd 30K is a glycoprotein present in small amounts in the 7S fraction of soy proteins. It has a molecular weight of 42.75 kDa and an isoelectric point of 5.79.

According to research, more than half of patients with soybean allergy are associated with an inadequate immune response to the epitope of this protein. It is considered the most allergenic of the soybean storage proteins and is the most thoroughly studied soybean allergen to date. Gly m Bd 30K is also known as P34 protein because of the similarity of its N-terminal peptide structure and composition to P34. In the work of Helm et al. **[25]**, who used the epitope mapping method, found that the main epitopes of Gly m Bd 30K are in protein segments 310339, 299-308, 229-238, 110-119 and 3-12. The ability of different IgE epitopes to bind varies between sera from different allergic patients. The alpha-helix is a key secondary structure for cross-reactive IgE/G epitopes; many of these epitopes are in the Nt domain and are composed of α-

helical structures **[26]**. Due to its homology with other allergenic proteins, P34 can cause cross-reactivity with milk casein from some ruminants and the pollen allergen from birch trees Bet v 1 **[27]**.

One of the main allergenic factors among soy proteins is a member of the 7S-globulin family known as Gly m Bd 28K, similar to its homologue with the number 30K. Its molecular architecture includes the predicted signal peptide Gm28K and a functional peptide with a molecular weight of 26000 Da **[28]**. The native molecule of the protein itself has a mass of only 2000 Da higher than that of the functional peptide and an isoelectric value of 5.62. The agent demonstrates a slightly reduced allergenic potential compared to Gly m Bd 30K, which correlates with its lower abundance in the soybean proteome. In the study by Kutateladze et al. **[29]** the main antigenic determinant of Gly m Bd 28K was identified: epitope 6 (^{256}SYNLYDDKKADFKNA^{270}), located on the periphery of the first β-layer of the cupin domain in the C-terminal region. The main terminal regions of the peptide chains responsible for adhesion to IgE are Y260, K264, D261 and D262. It has been reported that Gly m Bd 28K and P34 have a similar glycoprotein structure, and their glycosylated regions may serve as epitopes recognised by IgE antibodies.

Physicochemical ways to reduce the immunogenicity of soybean components

Minimising the antigenic properties of soy proteins requires the implementation of a multimodal approach, the central aspect of which is the induction of structural transformations of protein molecules. The application of advanced technological solutions opens prospects for targeted modification of the conformation and physicochemical characteristics of proteins, which potentially leads to a significant reduction in their ability to initiate a cascade of allergic reactions. Heat treatment is widely used as a method of reducing allergenicity. Most soya products are heated before consumption. This destroys the spatial configuration of proteins, reducing their allergenic properties; at the same time, heating inactivates protease inhibitors, which increases the nutritional value of soya protein and facilitates its digestion by proteases.

Li et al. **[30]** noted that heat treatment significantly affects the antigenicity of β-conglycinin. As the temperature increased from room temperature to 140°C, the antigenicity gradually decreased. After treatment at 140 °C, the antigenicity of β-conglycinin decreased to 60.78%, which is significantly lower than the initial level of 95-97.2% without heat. Immunoblotting showed that although heat treatment reduced the antigenicity of β-conglycinin, it could not be eliminated. The antigenicity of Gly m 3 decreased to 86.1% and 40.8%, respectively, after ten and five minutes of exposure to 100°C. X.

Pi et al. **[31]** mentioned the experiment of isolating and purifying P34 protein, then subjecting it to heating in a boiling water bath. They determined that the heat treatment changed the secondary configuration of P34: after 5 minutes of boiling, the linear epitopes became accessible and the antigenicity of P34 slightly increased, but with further heating, the immunoreactivity of the protein decreased. Based on these data, it is reasonable to assume that heat treatment is not able to completely and efficiently reduce the immunogenicity of soy globulin epitopes, and certain allergenic proteins show significant resistance to heat; therefore, simple heating has little effect on their antigenicity and should be combined with other processing methods.

The ultra-high pressure food processing method involves exposing food to a pressure of over 100 MPa under sterile and hermetic conditions at a temperature of 15-25°C. During this treatment, the structure of native molecules, including proteins and enzymes, changes, which reduces the antigenicity of allergenic proteins while maintaining the original taste of the product. In recent years, the use of ultra-high pressure to reduce the allergenicity of soy products has attracted increasing attention. The study by Kerezsi et al. **[32]** also determined that after soya protein was treated at a high hydrostatic pressure of 300 MPa for 15 minutes, its allergenicity decreased by 48.6% compared to untreated isolate. However, no significant change in allergenicity was observed when the pressure was further increased to 400 MPa and 500 MPa ($p>0.05$).

Li et al. **[30]** found that ultra-high pressure significantly alters the secondary and spatial structure of the three-dimensional β-protein conglycinin, and its antigenicity varies with pressure level. At 400 MPa, the antigenicity of β-conglycinin reached a minimum value, decreasing by 45.30% compared to the untreated sample; at this point, the content of α-helices and β-turns showed a significant reduction in the structural motifs of the secondary level of organisation, conjugated with a fundamental transformation of the protein's spatial topology. However, when the pressure was further increased to 500 MPa and 600 MPa, the antigenicity of β-conglycinin increased rather than decreased.

Mulalapele and Xi **[33]** also observed a similar phenomenon when high hydrostatic pressure was applied to β-conglycinin. The mentioned is probably because excessive pressure again alters the structure of the protein, exposing previously hidden antigenic epitopes, and resulting in increased antigenicity. As pointed out by Yao et al. **[34]**, the antigenicity of soya

globulin (11S) decreased after ultrahigh-pressure treatment, and the degree of decrease increased with increasing pressure. At 500 MPa for 20 minutes, the antigenicity reached a minimum level of 54.77%, but it increased again when the pressure was increased to 600 MPa. Thus, when using the ultra-high-pressure method, it is necessary to exercise high-grade control over the pressure and exposure time to reduce protein allergenicity more effectively. Enzymatic proteolysis is one of the priority methods in the spectrum of protein modification technologies, in which the spatial configuration of a protein macromolecule is restructured under the influence of specific biocatalysts. The resultant effect of this biochemical process is the disintegration of large protein structures into oligopeptide fragments or free amino acids, which is associated with a significant reduction in their immunogenic characteristics **[35]**.

Wang et al. **[36]** determined that during the hydrolysis of soy protein using a single enzyme (alkaline protease), part of the 11S-globulin was degraded, and the α- and α'-subunits of the 7S-globulin were hydrolysed. However, the β-component was partially preserved, although the antigenicity of both fractions decreased. When two enzymes (alkaline protease and papain) were used together, the 7S- and 11S-globulin subunits were rapidly degraded, and the decrease in antigenicity was more pronounced compared to the use of one enzyme.

Studies by El Mecherfi et al. **[37]** have used different types of proteases, including papain, neutral protease, bromelain, complex protease, taste protease, trypsin and alkaline protease, to hydrolyse the allergenic soybean protein P34. Molecular dynamics observations recorded a decrease in the level of P34 protein after treatment with all seven enzymes used, with taste protease and alkaline protease having the strongest effect, eliminating its immune activity under optimal hydrolysis conditions. In the experiment of Shu et al. **[38]** soybean product was hydrolysed using four enzymes: alkaline protease, pepsin, trypsin and taste protease. Structure-function analysis showed that all four enzymes were capable of hydrolysing soy proteins, with the highest degree of hydrolysis for alkaline protease and the lowest for trypsin. Analysis by polyacrylamide gel electrophoresis with sodium dodecyl sulfate revealed that the trio of enzymes, except trypsin, demonstrated catalytic potential for fragmentation of the corresponding number of subunits of the 7S-protein complex, along with the acidic and basic polypeptide chains of the 11S-protein. The ELISA method used to evaluate the antigenicity of hydrolysed proteins revealed that after hydrolysis by all four enzymes, the antigenicity of soy protein decreased to varying degrees. However, it was also noted that the hydrolysis process produced new peptides with a certain resistance to further cleavage.

Calcinai et al. **[39]** demonstrated that the synergistic use of alkaline and neutral proteases in an equimolar ratio for the proteolysis of soy protein isolate induced a more pronounced reduction in allergenic potential compared to the monoenzymatic approach. The integration of the above empirical data suggests that enzymatic hydrolysis effectively attenuates the immunogenicity of soy protein, and the multi-enzyme strategy potentiates this effect. Nevertheless, proteolytic degradation can be accompanied by the generation of oligopeptides with a pronounced bitter taste, which negatively affects the organoleptic characteristics of the final product.

Biomodification of soybean components: from glycosylation to genetic procession

The glycosylation process is a complex biochemical phenomenon consisting of the formation of covalent bonds between carbohydrate components and unbound amino acid fragments of protein molecules, resulting in the synthesis of glycoproteins. This reaction can induce the aggregation of protein structures and modification of antigenic determinants, which inevitably entails a transformation of the protein conformation and, as a result, its antigenic properties **[40]**.

The thermal effect accompanying the process can also act as a factor modulating the epitope configuration. The formation of neostructures during glycosylation has the potential to modify the allergenic potential of a protein by masking immunoglobulin E binding sites. Empirical studies have shown that exposing a soy protein isolate to glycosylation with fructose and fructooligosaccharides in the Maillard reaction resulted in a reduction in its allergenicity by almost 90% **[41]**. Similarly, glycosylation of β-conglycinin with glucose demonstrates a significant reduction in its antigenic properties **[42]**.

Yang et al. **[43]** determined that a complex of soy protein isolate and dextran in a mass ratio of 3:1 could be synthesised, and the antigenicity of the resulting glycosylated product was assessed by ELISA. Structural-functional analysis revealed an inverse correlation between the duration of the glycosylation reaction and the antigenicity of the product. After 6 days of the reaction period, a decrease in the immune activity of glycine and β-conglycine by 18.12% and 36.90%, respectively, compared to the parameters of the original sample was observed. Notably, β-conglycinin, being originally a glycoprotein, demonstrates a more pronounced susceptibility to glycosylation, which is

reflected in a more significant decrease in its antigenic properties. Further analysis using infrared spectroscopy revealed that the incorporation of carbohydrate chains induces conformational metamorphosis of macromolecules, manifested by the reduction of β-turns and disordered structures. The epitopes of the β-protein conglycinin, its α-subunit, were partially reduced during the above metamorphoses, which correlated with a decrease in its antigenicity. However, despite its potential effectiveness, the glycosylation process is characterised by a significant time duration and a limited effect on reducing the allergenicity of soy protein, which significantly limits the prospects for large-scale implementation of this methodology in practice. Enzymatic biotransformation is a complex process of utilising the metabolic activity of microorganisms under aerobic or anaerobic conditions to produce microbial biomass and primary or secondary metabolites. This methodology, being a traditional biotechnological paradigm in the food industry, has a wide range of applications. Fermentation products contribute to the organoleptic optimisation of food substrates without compromising their nutritional value; moreover, the fermentation process not only increases the bioavailability of nutrients but also has an inhibitory effect on pathogenic microflora.

In the experimental study by Rui et al. **[44]** an ensemble of eight strains of *Lactobacillus plantarum* (ZR, Y-1, MB1-6, L3-4, 70810, M-6, B1-6, and Dong) was used for the enzymatic degradation of isolated soybean protein. Subsequent analysis revealed a significant reduction in the antigenicity of the soy protein isolates in all experimental groups. This phenomenon indicates a potential degradation and modification of the epitope structures of soy protein during fermentation, which correlates with a partial reduction of its antigenic properties.

According to Venkataratnam et al. **[45]**, submerged fermentation of isolated soy protein using four microbial strains – *Bacillus subtilis*, *Rhizopus oryzae*, *Lactobacillus helveticus* and *Saccharomyces cerevisiae* – induces a significant reduction in the immunoreactivity of soy protein, which was verified by in vitro sandwich ELISA and immunoblotting. A particularly pronounced effect was observed during fermentation with *Lactobacillus helveticus*, reaching a 100% reduction of immunoreactivity.

In an experiment by Yang et al. **[46]** a consortium of three different microorganisms – *Lactobacillus casei*, yeast cultures and *Bacillus subtilis* – was used for solid-phase fermentation of soybean substrates. The post-fermentation allergenicity assessment showed a significant reduction in this parameter compared to the original substrate. A holistic assessment of the results of the above-mentioned experimental studies was used to postulate a significant probability of the effectiveness of enzymatic bioconversion in terms of minimising the allergenic potential of soy proteins. Nevertheless, it is necessary to note the outstanding specificity of fermenting microorganisms and the variability of their proteolytic activity towards soy proteins. From the perspective of industrial implementation of this methodology, it is critical to select and cultivate microbial cultures that demonstrate a pronounced ability to reduce the allergenicity of soy proteins.

The methodological arsenal of breeding includes mutagenesis, haploid and polyploid selection, hybridisation, and cell engineering methods. In the context of reducing the allergenic potential of soy protein, modern breeding approaches are differentiated into genetic engineering and non-genetic methodologies **[47]**. Breeding strategies aimed at modifying soy protein are characterised by a high safety profile; compared to physicochemical methods, breeding ensures the most efficient elimination of allergenic components from soy protein. An analysis of relevant literature shows that the use of hybrid breeding methods has made it possible to create new soybean varieties with a minimum content of α-, α′- and β-subunits of β-conglycinin **[48]**.

Sun et al. **[49]**, using the variety "Dongnong 47" as a maternal line and a line lacking the α′-subunit of the allergenic protein as a paternal line, obtained 12 new soybean lines characterised by the absence of the α′-subunit of 7S-globulin through hybridisation and autogamy. Hui et al. **[50]** used parental forms, maternal lines and eight breeding lines with eliminated subunits of 7S and 11S globulins as experimental material. The results of the study demonstrated a significant improvement in the agronomic characteristics of plants of lines with missing subunits compared to the original forms. Moreover, the absence of certain subunits induced a modification of the organoleptic properties of soybeans and a significant increase in the content of free lysine.

The use of genetic engineering methods to modify soy protein opens opportunities to eliminate genes responsible for allergenicity, thereby reducing the allergenic potential of soy protein. Genetic engineering technology, also known as recombinant DNA technology, allows for the artificial introduction of genes into recipient cells and their subsequent expression to produce targeted products. Ishibashi et al. **[51]** constructed a bifunctional RNA interference (RNAi) expression vector for the α′- and β-subunit genes of β-conglycinin and introduced it into the soybean plant of the Jinun 28 variety. PCR verification (polymerase chain

reaction) showed integration of the constructed bifunctional RNAi vector into the genome of transgenic soybean, which was accompanied by suppression of mRNA expression of α'- and β-subunit genes in the progeny. The ELISA revealed a 46.8-66.09% (w/w) decrease in β-conglycinin content in grains.

Yu et al. **[52]** conducted a comparative analysis of the immunogenic potential of allergens in transgenic and conventional soybeans. Experimental data did not reveal any deviations in the characteristics of the immune response of Gly m Bd 30K in transgenic and non-transgenic soybeans, but activation of Gly m Bd 28k transcription in transgenic soybeans was noted. At the same time, there is currently no consensus in the scientific community on the safety of genetically modified crops, which limits the widespread use of genetic engineering methods in this area.

Thus, a comprehensive characterisation of the key soybean allergens and innovative methodological approaches to their structural modification aimed at reducing the antigenic potential was presented. Although a comprehensive empirical database for each of the aspects discussed is still being developed, the development and optimisation of the described strategies represent a critical step in the context of global efforts to minimise the allergenicity of soy products. In this regard, the comparative analysis and systematisation of existing data carried out in this study not only contribute to a deeper understanding of soy allergy but also lay the foundation for further research and practical developments in this area.

Limitations

Despite the results achieved, there are still significant gaps in understanding the mechanisms of the immune response to processed proteins, as well as the long-term effects of processing technologies to reduce allergenicity. One of the unresolved issues is the impact of processed proteins on immune responses associated with cross-allergy, which requires additional research. There are also open questions regarding changes in the nutritional value and organoleptic properties of foods subjected to intensive processing. Promising areas for further research include the development of new technologies that provide a deeper modification of allergenic proteins with minimal impact on nutritional value. Another important area is the use of genetic engineering methods to create soybean varieties with reduced expression of allergenic proteins, which could be a breakthrough in the prevention of food allergies.

Conclusion

The study determined that the main allergens in soya products are glycine and β-conglycinin, which are high-molecular-weight proteins with a pronounced immunogenic potential. Both proteins have complex epitopes that are not always destroyed by heat treatment, which makes them highly resistant to inactivation processes. These proteins can cross-react with other allergens, which can aggravate allergic manifestations in patients predisposed to hypersensitivity not only to soy products but also to other plant proteins. Heat treatment was not effective enough to completely reduce the antigenic properties, although it did lead to a partial reduction in allergenicity. A more significant reduction in the immunogenic potential was achieved by applying ultra-high pressure, which causes pronounced structural changes in proteins, but this method also does not eliminate allergenicity. The highest efficiency was observed with the combined use of enzymatic hydrolysis and ultra-high pressure, which confirms the high role of enzymatic breakdown in the destruction of allergenic proteins into peptides and amino acids, significantly reducing the ability to cause immune reactions. Nevertheless, these methods do not guarantee complete inactivation of all epitopes capable of binding to immunoglobulins E, which highlights the need for further search for more effective solutions.

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The authors declare no conflict of interest.

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